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Subclinical myocardial disease by cardiac magnetic resonance imaging and spectroscopy in healthy HIV/Hepatitis C virus-coinfected persons

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Abstract

Objective: The contribution of hepatitis C virus (HCV) infection to the risk of heart failure in human immunodeficiency virus (HIV)-coinfected persons is unknown. The objective was to characterize cardiac function and morphology in HIV-treated coinfected persons.

Methods: In a cross-sectional study, HIV-infected patients virologically suppressed on antiretroviral therapy without known cardiovascular disease or diabetes mellitus underwent cardiac magnetic resonance imaging and spectroscopy for measures of cardiac function, myocardial fibrosis, and steatosis.

Results: The study included 18 male patients with a median age of 44 years. Of these, 10 had untreated HCV coinfection and eight had HIV monoinfection. Global systolic and diastolic function in the cohort were normal, and median myocardial fat content was 0.48% (interquartile range 0.35–1.54). Left ventricular (LV) mass index and LV mass/volume ratio were significantly greater in the HIV/HCV-coinfected group compared with the HIV-monoinfected group. In the HIV-monoinfected group, there was more myocardial fibrosis as measured by extracellular volume fraction.

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Conclusions: There were differences between HIV/HCV-coinfected and HIV-monoinfected patients in cardiac structure and morphology. Larger studies are needed to examine whether HIV and HCV independently contribute to mechanisms of heart failure.

Keywords
HIV, hepatitis C, cardiac magnetic resonance imaging, magnetic resonance spectroscopy, heart disease, myocardial fibrosis, myocardial steatosis

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Introduction

Human immunodeficiency virus (HIV) infection is associated with an increased risk for cardiovascular disease (CVD) complications, including myocardial, pericardial, and vascular disease.[1] The observed increased risk is mediated by various mechanisms, including HIV replication and the associated immune activation and inflammation, complications of antiretroviral therapy (ART) including metabolic toxicities such as dyslipidemia, insulin resistance and lipodystrophy, and higher rates of substance use and smoking.[1] The association between hepatitis C virus (HCV) infection and CVD is less well established, but suggested by an increasing number of studies to date.[2–4] While most investigations in HIV and HCV have focused on atherosclerotic disease and ischemic heart disease, a higher risk of heart failure has been reported even in the absence of pre-existing coronary artery disease.[5,6] HIV and HCV infection, which differ in terms of the interactions of their viral life cycles with host lipid metabolism and immune modulation, may distinctly influence risk for CVD, including risk of heart failure.

Accumulating but limited data in HIV-infected persons, primarily by echocardiography and only recently by cardiac magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS), suggests significant left ventricular (LV) dysfunction, left ventricular hypertrophy (LVH), myocardial fibrosis, and myocardial steatosis (intramyocardial triglyceride content) in HIV-infected persons without known CVD, all of which may be associated with future risk of clinical heart failure.[7–11] In previous studies, confounding by HIV viremia was inconsistently accounted for and the impact of virologic suppression upon these findings is unknown, given persistent HIV viremia is associated with greater immune activation and inflammation, which are thought to be major drivers of HIV-associated CVD.[7,8] Even more limited are the data examining the association between HCV and cardiac abnormalities, but the existing literature suggests an association between HCV and altered LV morphology, perhaps mediated by HCV-associated metabolic changes.[12,13] Lastly, there are no prior data on HIV/HCV coinfection and cardiac morphology. As treated and virologically suppressed HIV-infected persons are already at increased risk of CVD and current therapeutic options are limited to traditional approaches to CVD risk modification, if HCV coinfection independently promotes alterations in cardiac morphology and function, HCV itself would be a potential therapeutic target to reduce risk for CVD in HIV. HCV treatment today with interferon-free regimens is highly effective and well tolerated, yet access to treatment is often still limited due to cost, with priority given to those with more advanced liver disease.[14] Reducing CVD risk may be an argument for earlier treatment of HCV

regardless of liver disease stage, particularly in aging HIV-infected patients for whom CVD mortality is increasing.[15]

The aims of this pilot study were to characterize cardiac function, fibrosis, and steatosis by cardiac MRI and MRS in a virologically suppressed asymptomatic HIV cohort without known CVD, and explore differences between HIV/HCV-coinfected and HIV-monoinfected patients, where there may be distinct mechanisms for cardiac dysfunction and morphologic changes.

Patients and methods

Study design and patient selection

Study participants at least 18 years of age and without known CVD or diabetes mellitus were recruited for this cross-sectional study through advertisement and direct referrals from university-affiliated and local community-based HIV clinics and service providers, and enrolled at a single site, the University of California, Los Angeles Center for Clinical AIDS Research and Education, Los Angeles, CA, USA between May 2012 and May 2014. All patients had chronic HIV-1 infection and were on stable ART for at least 6 months with HIV viral load < 200 copies/ml and CD4 cell count $>$ 200 cells/mm^3. All HCV-coinfected patients were required to be naïve to HCV treatment and have HCV RNA $> 10{,}000$ IU/ml within 90 days prior to study entry. HCV-negative patients were required to have documentation of negative HCV serology within 1 year prior to study entry. Persons with chronic non-HCV liver disease, including coinfection with hepatitis B, were excluded. All participants were required to have glomerular filtration rate of ≥ 60 mL/min calculated by the Modification of Diet in Renal Disease equation within 45 days prior to entry.[16]

This study was reviewed and approved by the University of California, Los Angeles Institutional Review Board (IRB; protocol IRB#11-003092), and written informed consent was obtained from each study participant. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the UCLA IRB.

Clinical assessments

Standardized case report forms were used to collect demographic data, duration of HIV infection, smoking, substance use (cocaine, heroin, and methamphetamines), and alcohol use history, nadir CD4 cell count, co-morbid conditions, and concomitant medications including complete ART history. Resting blood pressure (the mean of two measurements), weight, height, and waist circumference were measured at study entry. Participants were required to be fasting for at least 8 h for fasting blood lipid panel and glucose measurements. Complete blood counts (CBC) and chemistries including hematocrit, creatinine, and hepatic transaminases (LFTs), HIV viral load, and HCV viral load were collected or abstracted, if available, from the medical record (CBC, LFTs, and HIV RNA within 180 days, HCV RNA within 90 days, and creatinine within 45 days of study entry). Hepatic fibrosis was estimated by the FIB-4 equation: [age (years) x aspartate aminotransferase (U/l)]/[platelet count (10^9/l) x [alanine aminotransferase (ALT, U/l)]$^{1/2}$.[17] ART was categorized by current ART (integrase inhibitor, protease inhibitor [PI], or non-nucleoside reverse transcriptase [NNRTI]-based and type of nucleos(t)ide reverse transcriptase, tenofovir plus emtricitabine or abacavir plus lamivudine) and a history of exposure to PI and NNRTI therapy.

Cardiac magnetic resonance imaging and magnetic resonance spectroscopy

Magnetic resonance imaging and ^{1}H MRS testing was performed during a single visit on a 3.0 Tesla (3.0 T) Siemens Trio

(Siemens Medical Solutions, Erlangen, Germany), using a phased array body coil. For LV function evaluation, a stack of short axis cine images as well as 2-, 3-, and 4-chamber views using steady state free precession imaging were acquired. Global heart function, including myocardial mass, volume, cardiac output, and ejection fraction (EF) were measured through contouring of the left ventricle using QMass® (Medis, Leiden, The Netherlands). To assess regional heart function, LV myocardial MR tagged images were obtained with three short-axis scan planes (10 mm thickness; gap of 5 mm) around the midlevel of the LV myocardium. The tagged MR images were analyzed using HARP® (Diagnosoft, Morrisville, NC, USA) to obtain measures of circumferential strain and strain rate. The strain relaxation index (SRI) was also calculated from the strain and strain rate curves as detailed previously.[18] An ECG-gated phase contrast gradient-echo sequence with velocity encoding was performed to measure blood flow across the mitral valve for determination of LV diastolic function. The resulting biphasic diastolic inflow pattern consisted of two peaks, representing the early filling phase and the atrial contraction. Analysis of the early filling phase and the atrial contraction were performed by calculating their peak filling rates and ratio of the peak filling rates (E/A) using QFlow® (Medis). Modified Look-Locker inversion recovery (MOLLI) sequence was used for T1 mapping before and 10 and 25 min after injection of 0.2 mmol/kg of gadopentetate dimeglumine. Long-axis images were acquired with TR/TE/flip angle = 3.9 msec/1.95 msec/50°, FOV = 360x360 mm, matrix = 240x192, and slice thickness = 8 mm. Late (15-min delay) gadolinium enhancement (LGE) images were acquired to assess for focal myocardial scar, in the same planes as the cine images. Extracellular volume (ECV) fraction, as the primary measure of diffuse myocardial fibrosis, was derived from the T1 values within the septal myocardium, LV blood pool and patients' hematocrit, calculated as the partition coefficient x (1-hematocrit).[19] Using all pre- and post-contrast T1 times, partition coefficient was calculated as the slope of the (fitted) line of the pre- and post-contrast myocardium R1 (1/T1) plotted against the pre- and post-contrast R1 of blood.[20] Both ECV and LGE images were analyzed using QMass® (Medis). The 4-chamber cine images were used for the measurement of epicardial and pericardial adipose tissue area as described previously.[21] The measurements were made in the end-diastolic phase using a ClearCanvas DICOM image viewer (Synaptive Medical, Toronto, Canada).[22] Epicardial fat was characterized as the areas of high intensity layer between the myocardium and the visceral pericardium, and pericardial fat as that outside the parietal pericardium. The short-axis cine images acquired were also used to guide in delineation of the areas. Subcutaneous and visceral fat volumes were measured from three transverse slices of the body at the L4–L5 level by semi-automated segmentation of axial images from a single-shot spin echo sequence.

^{1}H MRS protocol and analysis

Cine images of the left ventricle were used to position the spectroscopic volume (6-ml voxel) within the interventricular septum. Patients were instructed to breathe normally during spectroscopy. Two MRS spectra were recorded with electrocardiogram (ECG)-gated single voxel point-resolved spectroscopy sequence, with navigator across the liver-lung interface to reduce breathing effects. One spectrum (32 averages) was recorded with WET water suppression and another spectrum (eight averages) was recorded without water suppression. Outer volume suppression using saturation bands was applied to exclude the

lipids from ventricular blood flow and epicardial triglycerides. The same imaging sequence was also applied for ^{1}H MRS of the liver, utilizing an 8 ml voxel positioned within the liver, avoiding obvious vessels and the edge of the liver. Magnetic resonance user interface (MRUI) software (Jana Starčuková, Institute of Scientific Instruments, Academy of Sciences of the Czech Republic, Brno, Czech Republic) was used to process data.[23] The area under signals from methylene and methyl groups of fatty acids in triglycerides was quantified using the Advanced Magnetic Resonance fitting algorithm (Amares) and related to water in unsuppressed spectra.[24] Myocardial and hepatic triglyceride content were directly expressed as a percentage of fat to water signal ratios.

Statistical analyses. All patients with cardiac MRI and MRS data were included in the statistical analyses. Cardiac MRI and MRS were read by two investigators (C-Y.L. and B.A.) who were blinded to the HCV status of the participants. The statistical analyses were pre-specified in a data analysis plan. Summary data are presented as median and interquartile range (IQR) for continuous variables and as proportions for categorical variables. Given the small sample size, categorical variables were compared using Fisher's exact tests and continuous variables using non-parametric (Wilcoxon) methods. Bivariate regression models were used to explore associations between baseline clinical characteristics and radiographic or spectroscopic measures, including the association between myocardial fat and diastolic strain rate, liver fat, duration of HIV infection, visceral fat (corrected for body surface area [BSA] by the DuBois formula),[25] triglycerides, glucose, smoking status (current versus former and smoking pack-years), duration of ART, and type of current ART; ECV and diastolic strain rate, SRI, peak circumferential systolic strain (Ecc), and FIB-4; diastolic strain rate or Ecc and cocaine use (ever versus never), current CD4, duration of HIV infection, and type of current ART; Ecc and end diastolic mass (EDM) index (EDM/BSA) and EDM/end diastolic volume (EDV); diastolic strain rate and EDM index and EDM/EDV; SRI and EDM index and EDM/EDV; and mitral valve E/A ratio and ECV, EDM index, and EDM/EDV ratio. As this was an exploratory study, sample size calculations were not performed prospectively and adjustments were not made for multiple comparisons. All statistical analyses were performed using the SAS® statistical package, version 9.4 (SAS Institute Inc., Cary, NC, USA). A two-sided P-value ≤ 0.05 was considered statistically significant.

Results

Eighteen patients, all male, 10 with HIV/HCV coinfection and eight with HIV only, underwent cardiac MRI and MRS and were included in the analysis. Two HIV/HCV-coinfected patients did not have evaluable MRS and were excluded from the MRS analyses. Table 1 summarizes the baseline characteristics and MRI and MRS indices of the study cohort. Overall, a minority had traditional risk factors for CVD such as hypertension and dyslipidemia, but there were high rates of current or prior smoking and substance use, particularly alcohol and prior stimulant use. Of six participants on lipid-lowering therapy, three were on 3-hydroxy-3-methyl-glutaryl-Co-A reductase inhibitors, one was on niacin, and two were on fish oil or flax seed daily. HIV/HCV-coinfected and HIV-monoinfected patients were similar in most demographic, behavioral, and clinical characteristics relevant to cardiovascular risk. While smoking status examined by current or past smoking history was similar between the two groups, HIV/HCV-coinfected patients had a greater number of reported smoking pack years,

Table 1. Clinical and cardiac magnetic resonance imaging and ^{1}H magnetic resonance spectroscopy characteristics of the cohort overall ($n = 18$) and stratified by hepatitis C virus (HCV) status.

Characteristic	Overall cohort $n = 18$	HIV/HCV-coinfected group $n = 10$	HIV-monoinfected group $n = 8$	Statistical significance[a]
Age, years	44.0 (39.0, 48.0)	45.5 (41.0, 50.0)	43.0 (31.5, 47.0)	NS
Race				
White	6 (33)	3 (30)	3 (38)	NS
Black	8 (44)	5 (50)	3 (38)	
Other	4 (22)	2 (20)	2 (25)	
Body mass index, kg/m^2	26.3 (23.9, 29.1)	28.6 (25.7, 29.4)	24.1 (22.0, 26.3)	$P = 0.03$
Waist circumference, cm	89.2 (88.0, 98.7)	98.0 (89.0, 101.3)	88.8 (87.0, 89.0)	NS
Hypertension	4 (22)	2 (20)	2 (25)	NS
Systolic blood pressure, mmHG	116.3 (112.5, 124.0)	115.3 (113.0, 119.0)	116.8 (111.8, 127.5)	NS
Diastolic blood pressure, mmHG	71.0 (67.0, 78.0)	71.5 (71.0, 76.0)	68.0 (64.0, 85.5)	NS
Dyslipidemia	7 (39)	3 (30)	4 (50)	NS
On lipid-lowering therapy	6 (33)	2 (20)	4 (50)	NS
Alcohol status				
Current	11 (61)	5 (50)	6 (75)	NS
Former	5 (28)	4 (40)	1 (13)	
Never	2 (11)	1 (10)	1 (13)	
Smoking status				
Current	8 (44)	5 (50)	3 (38)	NS
Former	7 (39)	4 (40)	3 (38)	
Never	3 (17)	1 (10)	2 (25)	
Smoking pack years ($n = 15$, current or former smokers only)	4.60 (1.43, 12.50)	10.00 (3.25, 13.50)	3.00 (1.43, 9.00)	NS
Cocaine use				
Current	0 (0)	0 (0)	0 (0)	NS
Former	10 (56)	6 (40)	4 (50)	
Never	8 (44)	4 (40)	4 (50)	
Years since HIV diagnosis	11.4 (5.9, 17.9)	8.9 (4.8, 19.4)	13.9 (7.4, 17.4)	NS
Years on ART	6.80 (2.10, 15.10)	6.25 (2.10, 15.20)	7.40 (4.90, 16.25)	NS
Current ART				
Ritonavir-boosted PI	6 (33)	4 (40)	2 (25)	NS
NNRTI	6 (33)	2 (20)	4 (50)	
Integrase inhibitor	5 (28)	3 (30)	2 (25)	
Integrase + NNRTI	1 (6)	1 (10)	0 (0)	
CD4 nadir ($n = 9$ for HIV/HCV-coinfected group, $n = 7$ for HIV-monoinfected group), cells/mm^3	303 (116, 420)	373 (300, 500)	172 (25, 350)	$P = 0.05$
Current CD4 cell count, cells/mm^3	753 (550, 887)	768 (679, 909)	557 (436, 887)	NS
ALT, U/l	39.0 (19.0, 88.0)	79.5 (43.0, 97.0)	22.0 (14.5, 34.5)	$P = 0.03$
Total cholesterol, mg/dl	158.5 (136.0, 186.0)	155.5 (136.0 174.0)	158.5 (138.5, 188.5)	NS
LDL-C, mg/dl	92.8 (69.5, 114.2)	87.4 (61.8, 114.2)	92.8 (78.9, 118.3)	NS
HDL-C, mg/dl	41.4 (36.6, 46.3)	38.7 (32.3, 46.3)	44.0 (36.8, 46.7)	NS
Triglycerides, mg/dl	96.0 (73.0, 119.0)	107.0 (88.0, 147.0)	79.5 (71.5, 109.0)	NS

(continued)

Table 1. Continued.

Characteristic	Overall cohort $n = 18$	HIV/HCV-coinfected group $n = 10$	HIV-monoinfected group $n = 8$	Statistical significance[a]
Fasting glucose, mg/dl	88.0 (85.0, 95.0)	88.5 (84.0, 95.0)	88.0 (86.0, 96.5)	NS
FIB-4 score	0.93 (0.63, 1.38)	1.05 (0.83, 1.36)	0.76 (0.60, 2.13)	NS
Myocardial fat ($n = 8$ for HIV/HCV-coinfected group), %	0.48 (0.35, 1.54)	0.45 (0.35, 1.19)	0.51 (0.33, 1.62)	NS
Liver fat ($n = 8$ for HIV/HCV group), %	1.93 (1.33, 4.28)	2.30 (1.38, 4.04)	1.75 (1.06, 4.76)	NS
EDM index, g/m^2	56.81 (48.22, 61.45)	60.75 (55.53, 63.51)	48.93 (45.56, 55.38)	$P = 0.05$
EDM/EDV, g/ml	0.72 (0.63, 0.75)	0.75 (0.72, 0.77)	0.60 (0.57, 0.69)	$P < 0.01$
ESV index, ml/m^2	33.70 (28.29, 40.74)	33.70 (28.29, 39.76)	33.63 (28.47, 45.12)	NS
EDV index, ml/m^2)	80.95 (74.54, 85.47)	78.97 (74.54, 84.88)	82.94 (75.30, 94.24)	NS
Mitral valve E/A	1.25 (1.01, 1.43)	1.23 (0.94, 1.43)	1.29 (1.08, 1.80)	NS
Diastolic strain rate	0.11 (0.09, 0.14)	0.11 (0.10, 0.14)	0.10 (0.06, 0.13)	NS
Strain relaxation index	1.43 (1.07, 2.18)	1.17 (1.07, 1.67)	2.09 (1.21, 2.95)	NS
Peak circumferential systolic strain, Ecc	−18.43 (−19.92, −15.60)	−17.70 (−19.92, −15.60)	−18.51 (−20.64, −16.90)	NS
Ejection fraction, %	57.93 (52.00, 63.16)	58.67 (52.00, 59.87)	57.86 (52.13, 63.79)	NS
Extracellular volume fraction	0.27 (0.25, 0.31)	0.25 (0.24, 0.26)	0.31 (0.29, 0.32)	$P < 0.01$
Epicardial fat, mm^2	1012.0 (837.0, 1201.0)	937.5 (837.0, 1201.0)	1048.0 (860.0, 1180.0)	NS
Pericardial fat, mm^2	719.5 (366.0, 1352.0)	822.5 (560.0, 1412.0)	504.0 (167.0, 994.5)	NS
Subcutaneous fat/BSA, ml/m^2	105.66 (68.23, 122.96)	114.11 (68.23, 123.56)	87.38 (70.17, 109.72)	NS
Visceral fat/BSA, ml/m^2	70.27 (53.19, 86.16)	71.99 (66.45, 86.16)	59.51 (49.47, 100.45)	NS

Data presented as median (interquartile range) or *n* of patients (%).

[a]Categorical variables were compared using Fisher's exact tests and continuous variables using non-parametric (Wilcoxon) methods.

HIV, human immunodeficiency virus; ART, antiretroviral therapy; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; ALT, alanine aminotransferase; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FIB-4 score calculated using: [age (years) x aspartate aminotransferase (U/l)]/[platelet count (10^9/l) x [ALT (U/l)]$^{1/2}$; EDM, end-diastolic mass; EDM index, EDM/BSA; EDV, end-diastolic volume; ESV, end-systolic volume; ESV index, ESV/BSA; EDV, end-diastolic volume; EDV index, EDV/BSA; E/A, ratio of the peak filling rates; BSA, body surface area; NS, no significant between-group difference ($P > 0.05$).

although this did not reach statistical significance. Notably, body mass index (BMI) was significantly higher in the HIV/HCV-coinfected group compared with HIV-monoinfected group ($P = 0.03$), falling in the overweight range and suggesting potentially greater metabolic disease despite similar cholesterol and fasting glucose levels. With regard to HIV disease, the overall group appeared quite healthy with current CD4 cell counts > 500 cells/mm^3, but the HIV/HCV-coinfected group had evidence of having initiated ART earlier in HIV disease with a higher CD4 nadir. The distribution of current ART class (ritonavir-boosted PI, NNRTI, and integrase) was similar between the two groups and only one subject (in the HIV/HCV-coinfected group) was on an abacavir backbone. All but one participant had HIV RNA < 48 copies/ml at baseline. With regard to liver disease, as expected, ALT was significantly higher in the HIV/HCV-coinfected group compared with the HIV-monoinfected group ($P = 0.03$); a single subject in the HIV/HCV-coinfected group had a diagnosis of cirrhosis, and the median FIB-4 score was low, suggesting overall low rates of hepatic fibrosis in the

study cohort. Hematologic indices (CBC and international normalized ratio) were normal (data not shown); and renal function was preserved in the cohort, although nonsignificantly lower in the HIV/HCV-coinfected group (median 86.5 versus 95.3 ml/min in the HIV-monoinfected group).

Overall median myocardial fat (%) was 0.48 (IQR 0.35–1.54) and similar between HIV-monoinfected and HIV/HCV-coinfected groups. Participants without a history of HIV PI exposure ($n = 5$) had three times the degree of myocardial fat of those with a history of PI use ($n = 12$), but this was not statistically significant (median [IQR] = 1.35% [0.35–1.90] versus 0.46% [0.26–0.64], respectively). Cardiac function measures suggested similar or worse diastolic function in the HIV/HCV-coinfected group compared with the HIV-monoinfected group and normal systolic function in both groups (Table 1). In unadjusted analyses, four (22%) patients had an abnormal mitral valve E/A ratio (<1), three of 10 (30%) in the HIV/HCV-coinfected group and one of eight (13%) in the HIV-monoinfected group, but the difference between the groups was not significant. Two patients in the HIV-monoinfected group had evidence of a focal myocardial scar, one at the inferior right ventricular insertion and the other in the basal anterolateral wall, suggesting prior ischemic disease.

There were notable morphologic findings in the cohort. Overall, ECV values were at the upper limit of normal and there was significantly greater myocardial fibrosis by ECV in the HIV-monoinfected group versus the HIV/HCV-coinfected group (median ECV 0.31 versus 0.25, respectively, $P < 0.01$; mean ± SD ECV 0.30 ± 0.02 versus 0.26 ± 0.03, respectively). Five of the 18 participants had an abnormal ECV > 0.30; four in the HIV-monoinfected group and one in the HIV/HCV-coinfected group. In contrast, there was significantly greater LV mass and EDM/EDV ratio in the HIV/HCV-coinfected group versus the HIV-monoinfected group ($P = 0.05$ and $P < 0.01$, respectively; Table 1). Subcutaneous and visceral fat volumes were greater in the HIV/HCV-coinfected versus the HIV-monoinfected group, but not significantly (Table 1).

There were significant associations of myocardial fibrosis and LV mass, but not myocardial steatosis, with select diastolic and systolic function measures, and significant associations between myocardial fat and other metabolic and fat deposition measures. Specifically, by bivariate regression including all patients, myocardial fat was not associated with diastolic strain rate or SRI. Degree of liver fat was associated with the degree of myocardial fat ($P = 0.013$, $\beta = 0.099$) and level of fasting glucose was a strong predictor of myocardial fat content ($P < 0.001$, $\beta = 0.095$) (Table 2). Visceral fat (indexed for BSA) approached statistical significance in its association with myocardial fat ($P = 0.059$, $\beta = 0.013$) and was significantly associated with epicardial fat ($P < 0.01$, $\beta = 4.99$), but not serum triglyceride level. Duration of HIV infection and ART and baseline ART type were not associated with the degree of myocardial fat. ECV was not associated with diastolic strain rate, nor with Ecc, but was associated with SRI ($P = 0.017$, $\beta = 0.157$). LV mass and EDM/EDV ratio were associated with systolic strain (Ecc) ($P = 0.036$, $\beta = 0.163$ and $P < 0.01$, $\beta = 18.69$, respectively), but not with diastolic strain measures, although near statistical significance for E/A ratio ($P = 0.07$, $\beta = -2.18$). Hepatic fibrosis by FIB-4 score was not associated with myocardial fibrosis (ECV). Additional bivariate analyses exploring substance use and HIV parameters as predictors of diastolic and systolic strain measures were not revealing, including absence of associations with history of cocaine use, duration of HIV infection, baseline CD4 cell count, and ART type.

Table 2. Bivariate linear regression for associations with cardiac steatosis and function in the overall cohort of study participants ($n = 18$).

Outcome	Predictor	Parameter estimate	Statistical significance
Myocardial fat, %	Age, years	0.056	$P = 0.012$
	Hepatic fat, %	0.099	$P = 0.013$
	Fasting glucose, mg/dl	0.095	$P < 0.001$
	Visceral fat/BSA	0.013	NS
	Serum triglyceride level, mg/dl	–0.0007	NS
	Duration of ART, years	0.042	NS
Strain relaxation index, ms	Age, years	0.021	NS
	ECV, %	0.157	$P = 0.017$
	EDM/BSA	0.033	NS
	EDM/EDV	–0.575	NS
Peak circumferential systolic strain, Ecc	Age, years	0.111	NS
	EDM/BSA	0.163	$P = 0.036$
	EDM/EDV	18.69	$P < 0.01$

BSA, body surface area; ART, antiretroviral therapy; ECV, extracellular volume fraction; EDM, end diastolic mass; EDV, end diastolic volume; NS, no significant association ($P > 0.05$).

Discussion

This pilot study of HIV-monoinfected and HIV/HCV-coinfected persons found signals of differences in cardiac structure and morphology by HCV status on comprehensive cardiac MRI imaging, with greater myocardial fibrosis in HIV-monoinfected persons and greater LV mass in HIV/HCV-coinfected persons. These morphologic differences suggest that HIV and untreated HCV infection, and their interaction, may alter the myocardium distinctly. Hypothesized mechanisms for these morphologic differences may include differences in viral infection-driven inflammation and metabolic dysfunction, such as insulin resistance, hepatic steatosis, lipid derangements, lipodystrophy and cardiac lipotoxicity. Both HIV and HCV are associated with increased risk for insulin resistance; in HIV, insulin resistance is associated with ART and lipodystrophy; and in HCV, where the association is well established but the mechanisms less well understood, it may relate to direct interference by HCV in insulin signaling.[26,27] Insulin resistance and hyperinsulinemia may independently promote myocardial cell proliferation and LV hypertrophy.[28] In this current study, the HIV/HCV-coinfected patients had more signs of metabolic abnormalities than the HIV-monoinfected patients, with higher BMI, waist circumference, triglyceride levels, and hepatic steatosis, although absent hypertension, suggesting that greater metabolic disease and insulin resistance from HCV coinfection could be driving the LV mass differences seen. Others have found greater LV mass in HCV-monoinfected patients similar to HCV-negative hypertensive patients, correlating with HOMA-IR.[12] To our knowledge, no studies have directly compared HIV/HCV-coinfected and HIV-monoinfected patients with regard to cardiac function or insulin resistance, and teasing out the relative contributions of each virus and their interaction may provide insight into expected cardiac complications and best management.

In this current cohort, diffuse myocardial fibrosis was greater in HIV-monoinfected

patients compared with HIV/HCV-coinfected patients, with a difference of 6% of the myocardium being affected. Amongst the HIV-monoinfected participants, half had ECV > 30%, which is generally considered to be abnormal.[29] Several studies have demonstrated the correlation of increasing ECV values across a range similar to those in this current study with impaired diastolic function and LV stiffness.[30,31] In one study, the difference in median ECV between participants with heart failure with preserved EF and without heart failure, and between those with systolic failure and without heart failure, was smaller than seen in this current study.[30] In another study of obese adults, the hazard ratio for hospitalization for heart failure was 1.92 (95% confidence interval [CI] 1.40, 2.65) for every 5% increase in ECV, and 2.50 (95% CI 1.59, 3.95) for death.[32] In that study, the risk of hospitalization for heart failure or death was increased in participants with ECV > 25% compared with those with ECV < 25%.[32] Thus, the 6% difference in ECV between HIV-monoinfected and HIV/HCV-coinfected patients in the present study is likely to be clinically significant; and in addition, three-quarters of all participants in the present study had ECV > 25%. Why ECV was greater amongst HIV-monoinfected compared with HIV/HCV-coinfected patients is unclear. One notable difference between the HIV-monoinfected and HIV/HCV-coinfected groups was a lower CD4 nadir in the monoinfected group, which may be associated with greater immune activation, inflammation, and fibrogenesis. However, in a larger cohort of HIV-monoinfected patients previously reported,[11] no association was seen between ECV and nadir CD4 or soluble biomarkers of inflammation and immune activation. Longer duration of untreated HIV infection in the HIV-monoinfected group leading to a greater chronic inflammatory state driving myocardial collagen deposition is also a consideration as a potential mechanism for greater ECV. The HIV-monoinfected group had a longer duration of time since HIV diagnosis (median of 13.9 versus 8.9 years, respectively) despite relatively similar duration on ART. Considering other associations with ECV, in a previous study, systolic blood pressure was associated with ECV but blood pressure was higher than in this current cohort, where systolic blood pressure was similar between coinfected and monoinfected participants and normal.[11] It could also be considered that differences in past or present ART and associated cardiotoxicity may have contributed to differences in myocardial fibrosis, but the limited size of this present study and of others to date preclude such examination. Larger studies will be needed to confirm if there is a difference in myocardial fibrosis between coinfected and monoinfected patients and to elucidate the etiology of myocardial fibrosis in HIV-infected patients without hypertension.

In the current study, ECV values in the HIV-monoinfected group were greater than, and in the HIV/HCV-coinfected group similar to, that observed in HIV-uninfected controls in a previous study that examined a US cohort with similar demographic and clinical characteristics to the cohort in the current study, with MR also conducted on a 3.0 T MR scanner.[11] In the current study, ECV values in the HIV-infected group were greater than those in the HIV-infected group in the previous study (mean ± SD 0.30 ± 0.02 versus 0.28 ± 0.04, respectively), where ECV values were statistically significantly greater than uninfected controls (ECV mean ± SD 0.26 ± 0.03).[11] In one European study that used a 1.5 T MR scanner, T1 mapping short MOLLI values were also significantly greater in HIV-infected compared with uninfected controls (ECV not reported);[7] while another smaller European study that used a 3.0 T MR scanner found no difference in ECV between

HIV-infected and uninfected persons.[33] Mean ECV values in HIV-uninfected controls in previous studies[11,33] and in a non-HIV study of healthy subjects compared with patients with different cardiac disease conditions[29] were similar. Thus, ECV values in uninfected healthy subjects without baseline CVD appear consistently in the 0.25–0.26 range and the higher ECV values in the HIV-infected patients in the current study may reflect a true increase from healthy myocardium due to either HIV infection or other CVD risk factors more prevalent in HIV. Further studies with carefully selected controls are needed to tease out the contribution of HIV itself to myocardial fibrosis.

While systolic and diastolic function were similar in HIV/HCV-coinfected and HIV-monoinfected patients in the current study, and overall were normal in this apparently healthy and relatively young, diverse cohort with well-controlled HIV and without overt metabolic disease, the alterations in myocardial fibrosis and myocardial mass with suggest the potential for future cardiac dysfunction and major cardiovascular events in HIV and HIV/HCV infected persons.

Overall, there was significant myocardial steatosis in the current cohort, similar to that seen in a previously reported HIV cohort,[7] where myocardial fat content was greater in HIV-infected persons compared with uninfected controls, but lower than that seen in another published study.[11] Similarly, less liver fat was seen in the present cohort than in this latter previously published study; in contrast, the degree of diffuse myocardial fibrosis by ECV was similar.[11] Some of these differences may potentially be explained by differences in the participants enrolled in the two cohorts.[11] For example, the cohort in the present study were younger by 5 years and healthier with less metabolic derangement, and none was women compared with the previous study, in which age, fasting glucose, triglyceride level, and female sex were associated with myocardial fat.[11] Compared with the previous cohort, none of the participants in the current study had diabetes mellitus or were female, and they had a lower BMI, fasting glucose, and triglyceride levels.[11] The current study participants also differed in HIV status: all had a suppressed HIV viral load compared with 84.2% of the previously reported cohort; although it should be noted that the viral load cut-off for a suppressed viral load differed between the two studies (50 copies/ml in the previous study versus 200 copies/ml in the current study).[11] The patients in the current study also had a higher reported nadir CD4 cell count compared with the previously reported cohort (303 versus 236 cells/μl, respectively).[11] Interestingly and in direct contrast to pre-study expectations, while not statistically significant, participants in the current study without a history of HIV PI use had three times greater myocardial steatosis content compared with participants with a history of PI use. Given the small sample size (five in the no PI group), this difference may have occurred by chance and not reflect a true difference related to ART exposure, but is striking and should be explored in larger studies. Other studies have either not assessed associations between specific ART type and myocardial steatosis or reported no association between specific ART subclasses and myocardial fat content, without providing specific values for each subgroup.[7,11] Understanding if specific ART exposure is associated with increased myocardial fat content may improve assessment of individual risk for cardiac dysfunction as well as understanding of mechanisms of intramyocardial lipid accumulation in HIV-infected patients. Myocardial steatosis has been implicated in the pathogenesis of LV hypertrophy, diastolic dysfunction, nonischemic cardiomyopathy, and overt heart failure.[34–37] The correlation between hepatic steatosis and myocardial steatosis, yet lack

of correlation between hepatic fibrosis and myocardial fibrosis, suggests potentially differing pathogenic mechanisms of end-organ fibrosis and the contribution of steatosis to fibrosis development in these organs.

Strengths of the current study include: (i) prospective enrollment of participants utilizing strict pre-specified eligibility criteria, thus limiting heterogeneity of the cohort; (ii) limited confounding by active HIV viremia as all participants were required to have documented HIV virologic suppression, which has been a limitation of previous studies of cardiac imaging in HIV-infected persons;[7,11] and (iii) utilization of standardized MRI and ^{1}H MRS protocols with blinding of MRI and MRS readers to the HCV status of participants, reducing bias or technical deviations. Significant limitations of the study include: (i) its small sample size, which limited the ability to explore associations between HIV and HCV-specific factors and the outcomes of interest and conduct adjusted analyses, such as to address potential differences between HIV and HIV/HCV groups in demographics, traditional cardiovascular risk factors, and HIV disease severity; (ii) inclusion of patients from a single site, limiting generalizability of the study; (iii) lack of matching of HIV and HIV/HCV patients, although they were similar in many key characteristics; and (iv) absence of an HIV-uninfected control group. Such a control group would allow assessment of the independent contribution of HIV and HCV to the risk of or unique mechanisms for cardiac abnormalities, particularly given that the cardiac MR measures are influenced by differences in technique such as field strength and pulse sequences used and region of measurement, and direct comparisons with other studies of HIV-uninfected persons may not be appropriate.[38]

In conclusion, despite the small sample size, this current study found evidence of expected relationships between cardiac abnormalities detected by MRI/MRS and systolic and diastolic function, including associations between myocardial fibrosis and diastolic dysfunction and LV mass and systolic dysfunction. The current study found similar associations as with larger studies utilizing cardiac MRI/MRS in HIV-infected cohorts, with differences in findings between this and other cohorts highlighting that the prevalence and degree of subclinical cardiac abnormalities, even in healthier HIV cohorts, will likely vary significantly depending on patient selection. Understanding the observed findings will require better characterization of the overlap between HIV, metabolic disease, and other co-morbidities such as viral hepatitis. Larger studies are needed to further explore these preliminary findings and to examine the effect of HIV and HCV on cardiac function with adjustment for potentially confounding demographic, behavioral, and clinical characteristics, as well as to assess the impact of HCV treatment on measures of cardiac function. Understanding differences in the pathogenesis of cardiac disease in HIV and HCV-infected persons will allow for improved clinical risk assessment for heart failure, arrhythmia, and sudden death, as well as identify pathways, such as cardiac fibrogenesis and hemodynamic alterations related to liver disease, and systolic versus diastolic dysfunction, which may be differentially targeted for the treatment or prevention of cardiac complications in HIV and HCV. There is a very high prevalence of HCV infection and coinfection globally.[39–41] Such affected patients are aging and at increasing risk for extra-hepatic and non-AIDS complications, with cardiovascular disease being a leading cause. The contribution of HCV infection to cardiovascular disease, and specifically the underexplored conditions of myocardial disease and heart failure, deserves further investigation.

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Declaration of conflicting interests

C-Y.L., B.A., D.L., T.B.H., J.A.C.L., D.A.B., J.P.F., and J.S.C. declare that they have no conflicts of interest. K.W.C. has received investigator-initiated grant support to the institution from Merck & Co. and research support to the institution from Gilead Sciences, unrelated to the current project. A.A.B. has received investigator-initiated grants to the institution from Gilead Sciences and AbbVie, unrelated to the current project.

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